Evolution of a new class of antihypertensive drugs- targeting the brain renin angiotensin system.

Catherine Llorens-Cortes[1 #] and Rhian M Touyz[2]

[1] Laboratory of Central Neuropeptides in the Regulation of Body Fluid Homeostasis and Cardiovascular Functions, Collège de France, Center for Interdisciplinary Research in Biology (CIRB), INSERM U1050/CNRS UMR 7241, 11 Place Marcelin Berthelot, 75005 Paris, France.

[2] Institute of Cardiovascular and Medical Sciences, University of Glasgow, United Kingdom

[#] To whom correspondence should be addressed. Collège de France, INSERM U1050, 11 place Marcelin Berthelot, 75231 Paris Cedex 05, France. Tel: + 33 1 44271663. Fax: + 33 1 44271691. e-mail: c.llorens-cortes@college-de-france.fr

Short title: Firibastat, a novel antihypertensive drug.

Key Words: Aminopeptidase A, zinc metalloprotease inhibitor, brain renin-angiotensin system, resistant hypertension

7586 words

ABSTRACT

Brain renin-angiotensin system (RAS) hyperactivity has been implicated in the development and maintenance of hypertension. Angiotensin III, generated by aminopeptidase A (APA), one of the main effector peptides of the brain RAS, exerts a tonic stimulatory control over blood pressure in hypertensive rats. This identified brain APA as a potential therapeutic target for the treatment of hypertension, leading to the development of RB150 an orally-active prodrug of the specific and selective APA inhibitor, EC33. When given orally, RB150 crosses the gastrointestinal and blood-brain barriers, enters the brain, generates two active molecules of EC33 which inhibit brain APA activity, blocking brain angiotensin III formation, and decrease blood pressure for several hours in hypertensive rats. The RB150-induced blood pressure decrease is due to a reduced vasopressin release, which increases diuresis, reducing extracellular volume, a decrease in sympathetic tone, leading to a reduction of vascular resistances and the improvement of the baroreflex function. RB150 was renamed firibastat by the WHO. Phase Ia/Ib clinical trials showed that firibastat is clinically and biologically well-tolerated in healthy volunteers. Clinical efficacy of firibastat in hypertensive patients was therefore demonstrated thanks to two Phase II studies. Overall, firibastat could represent the first drug of a new class of antihypertensive agents targeting the brain RAS.

Hypertension affects one third of the adult population and is the leading cause of premature death and disability-adjusted life years [1,2]. It is a major risk factor for many diseases including coronary heart disease, cerebral vascular accidents, cardiac failure and renal dysfunction. Despite existing therapy, hypertension remains poorly controlled worldwide [3], and its prevalence is increasing due to ageing of the population and the obesity epidemic [4]. The prevalence of hypertension among U.S. adults aged >18 years was 29% and even higher among Non-Hispanic black adults (40.6%) [5]. In African-Americans, hypertension is more severe with a higher morbidity and mortality than in Whites [6]. Among the many effective antihypertensive drugs currently used, are inhibitors of the systemic renin-angiotensin system (RAS). These include drugs that inhibit formation of angiotensin II (AngII) by angiotensin I -converting enzyme (ACE, EC 3.4.15.1), called ACE inhibitors (ACEI) and agents that block the action of AngII on type 1 AngII receptors (AT_1) by AT_1 receptor antagonists (ARB) [7]. However, secondary effects of ACE inhibitors, such as cough and more rarely angioedema have been observed [8]. Renal function may also deteriorate in underlying renal artery stenosis [9]. In addition, blockers of the systemic RAS are poorly effective in African-Americans in whom high BP is often accompanied by a low-renin state (decrease in systemic RAS activity) and high plasma arginine-vasopressin levels [6,10]. Monotherapy for hypertension treatment is ineffective in more than half of all cases and responses to a given compound, regardless of its chemical family, differ greatly between individuals. Most hypertensive patients require two or more antihypertensive drugs to control BP including a RAS blocker, a calcium channel blocker or a diuretic [11–13]. Adding to the challenges in controlling blood pressure (BP) in patients with hypertension, is the growing incidence of resistant hypertension (~15%) where at least three antihypertensive drugs (including a diuretic) are required [14,15]. Accordingly, there is a real clinical need to develop novel classes of antihypertensive agents acting on new targets, with diversified modes of action, to better manage BP control.

The brain renin-angiotensin system

In addition to the systemic RAS, the brain RAS plays an important role in the control of cardiovascular function and BP regulation [16–18]. All components of the systemic RAS, ie, the precursor angiotensinogen, the enzymes, renin (EC 3.4.23.15), angiotensin I-converting enzyme (ACE, EC 3.4.15.1), angiotensin converting enzyme type 2 (ACE2, EC 3.4.17.23), aminopeptidase A (APA, EC 3.4.11.7) and aminopeptidase N (APN, EC 3.4.11.2), the peptides AngI, AngII, AngIII, AngIV, Ang1-7 and the AngII receptors, type 1 (AT1), and type 2 AngII (AT2) receptors as well as the Mas receptor (MasR) are present within the brain (reviewed in [19–21]) (Figure 1).

Hyperactivity of the brain RAS has been implicated in the development and maintenance of hypertension and its interruption by either pharmacological or genetic means is associated with a profound beneficial outcome in hypertension [16,17]; its components could constitute interesting targets for treatment of hypertension.

Aminopeptidase A and Aminopeptidase N Inhibitors

AngII is hydrolysed to numerous peptides, which themselves may have functional significance. The best candidate enzymes involved in the hydrolysis of AngII and AngIII include two membrane-bound zinc-metallo-peptidases : APA and APN [22–24]. This is based on the observation that *in vitro* purified APA hydrolyses the N-terminal aspartate residue from AngII to generate AngIII[25] and that purified APN cleaves the N-terminal arginine of AngIII to generate AngIV [26].

To demonstrate that APA and APN are involved *in vivo* in the metabolism of brain angiotensins, specific and selective APA and APN inhibitors were required. APA inhibitors have been developed by rational design taking into account APA substrate specificity, APA exopeptidase activity and the presence of the Ca^{2+} and Zn^{2+} atoms in the APA active site [27]. Some of these potential inhibitors were based on the structure of the glutamate thiol (GluSH), which is a potent but non-selective inhibitor of APA and APN [28] . In this context, Chauvel *et al.* [29] designed

an APA inhibitor, EC33 ((S)-3-amino-4-mercapto-butyl sulfonic acid), in which the GluSH carboxylate side-chain was replaced by a sulfonate (Figure 4). This resulted in an increase of the polarity of the sulfonate side chain and in its interaction with the calcium ion, thus improving selectivity towards APA [29]. In addition, an effective APN inhibitor was developed: PC18 (2-amino-4-methylsulfonyl butane thiol) [30]. Pharmacological characterization of these inhibitors performed on purified APA and APN showed that EC33 inhibited APA (Ki = 0.29 µM) almost 100 times more strongly than APN. Conversely, PC18 inhibited APN (Ki = 0.008 µM) 2150 times more efficiently than APA (Ki = 17.2 µM) [30,31]. A systemically active prodrug of EC33, RB150 (4,4´-dithio bis[3-amino butyl sulfonic acid]) (Figure 4) was also developed. This inhibitor comprises two molecules of EC33 linked by a disulfide bridge. As the thiol group of RB150 is engaged in the disulfide bridge, it cannot inhibit APA enzymatic activity since it is unable to interact with the zinc atom present in the APA active site and essential for its catalytic activity. However, the disulfide bridge allows orally administered RB150/firibastat to cross the intestinal, hepatic and blood–brain barriers. On entry into the brain, it is cleaved by brain reductases to generate two active molecules of EC33. Moreover *in vitro*, the reduced form of RB150 obtained in the presence of dithiothreitol (DTT) inhibited purified APA (Ki = 0.20 ± 0.02 µM), similar to EC33 [32]. The selectivity of EC33 and RB150 toward APA was shown by the lack of affinity of these compounds for other zinc metalloproteases involved in the production or metabolism of vasoactive peptides, such as APN, ACE, ACE-2, ECE-1 and NEP, as well as by the absence of binding of these compounds to AT1 and AT2 or endothelin A and B receptors known to be involved in BP regulation [33].

More recently, a new more potent and selective central-acting APA inhibitor prodrug, NI956/QGC006, was developed [34]. This compound was obtained by disulfide bridge-mediated dimerization of NI929 ((3S,4S)-3-amino-4-mercapto-6-phenyl-hexane-1-sulfonic acid), a non-peptidic APA inhibitor that is 10 times more potent than EC33 at inhibiting recombinant mouse

APA activity *in vitro* (Ki value of 30 nM). NI956 did not inhibit other enzymes (ACE, ACE2) or receptors (AT1, AT2, endothelin type A and B, vasopressin type1 and type 2, apelin, bradykinin and urotensin II) involved in BP control when tested at 10^{-5} M.

Distribution of APA in the Brain in Parallel with AT1 Receptors

APA enzymatic activity measured in the absence or presence of the specific and selective APA inhibitor, EC33, has also been identified in rat brain nuclei involved in the control of body fluid homeostasis and cardiovascular functions [35]. The lowest and highest levels differed by a factor of 150. The pituitary gland and circumventricular organs were the richest source of this enzyme, followed by the median eminence, the arcuate nucleus, the choroid plexus, the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei, the area postrema (AP), the lateral reticular nucleus (LRN) and the nucleus of the solitary tract (NTS) (Figure 2C) [35]. There is a good relationship between the brain distribution of APA activity, angiotensin receptors [19,36] (Figures 2A-2B) and nerve terminals [37].

In the human brain, immunostaining of sections through the medulla oblongata shows a high density of APA immunoreactive neuronal cell bodies and fibers in the motor hypoglossal nucleus (XII), the dorsal motor nucleus of the vagus (X), the nucleus of the solitary tract (NTS), the Roller nucleus and the nucleus ambiguus [38] (Figure 2D). APA immunoreactivity was also visualized in vessels and capillaries in the dorsal motor nucleus of the vagus and the inferior olivary complex [38]. Thus, there is clear evidence demonstrating that brain APA may be associated not only with neuronal cells, but also with microvessels.

The presence of APA in several brain nuclei containing angiotensin nerve terminals and AT1 receptors involved in BP regulation suggests that APA is an integral component of the brain RAS in humans and rodents and further supports the idea that APA inhibitors could be clinically tested as an additional therapy for the treatment of certain forms of hypertension.

In Vivo Metabolic Pathways of Brain AngII and AngIII

Harding *et al.* were the first to investigate the metabolism of brain AngII and AngIII *in vivo* [39]. They reported that the half-lives of intracerebroventricularly (i.c.v) injected AngII and AngIII were approximately 23 and 8 seconds, respectively, indicating that AngIII is metabolized more rapidly than AngII. To block AngII and AngIII metabolism, they used the aminopeptidase inhibitors, amastatin and bestatin, and showed that the administration of both inhibitors i.c.v extended the half-lives of [^{125}I]AngII and [^{125}I]AngIII [40,41]. These results indicate that amastatin and bestatin are efficient blockers of angiotensin metabolism. However they are not selective with respect to AngII and AngIII. Consistent with this, amastatin, which was initially described as a specific APA inhibitor, actually inhibits APN 40 times more efficiently than APA, whereas bestatin displays broad specificity for various aminopeptidases [42,43]. *In vivo* studies showed that the APA inhibitor, EC33 (1–100µg) injected by i.c.v route in conscious mice inhibited brain APA activity in a dose-dependent manner with an IC_{50} of 12 µg [32]. EC33, i.c.v co-injected with radiolabeled AngII into conscious mice, completely blocked the formation of [^{3}H]AngIII in the hypothalamus [44], whereas the APN inhibitor, PC18, increased the half-life of [^{3}H]AngIII by a factor of four [31]. These results provided the demonstration that APA is involved *in vivo* in the formation of brain AngIII from AngII whereas APN metabolizes AngIII in AngIV.

Brain AngIII in the Control of Blood Pressure

The notion that the physiologically relevant peptide in the brain RAS responsible for the regulation of BP is AngIII rather than AngII was suggested in 2003 [45,46]. Since then many studies have supported the "AngIII hypothesis". AngII and AngIII display similar affinity for AT1 and AT2 receptors [47]. In addition, these peptides have a similar affinity for a non-AT1, non-AT2 angiotensin binding site which shares some characteristics with the liver cytosolic AngII binding proteins, later identified as EC 3.4.24.15 (thimet oligopeptidase) and/or EC 3.4.24.16 [48]. Harding *et al.*

159 demonstrated, in a push–pull cannula study, that 93% of the angiotensin material released in the
160 PVN, after simulation with veratridine or by water deprivation, was in the form of AngIII whereas
161 only 6.8% corresponded to authentic AngII [49]. AngII and AngIII, when centrally administrated,
162 similarly increase BP, stimulate vasopressin release and decrease baroreceptor reflex function
163 [20,44,50–52].

164 AngII and AngIII increase BP by three mechanisms: (i) increase in sympathetic nerve activity, (ii)
165 synaptic inhibition of the baroreflex in the nucleus of the tractus solitarius, and (iii) release of AVP
166 into the bloodstream [53].To determine the respective roles of AngII and AngIII in the central control
167 of BP, the effects of AngII and AngIII, injected by i.c.v route, on BP in hypertensive rats were
168 studied in the absence and presence of APA or APN inhibitors, EC33 and PC18 respectively. Two
169 experimental models of hypertension were used: the SHR, a genetic model of hypertension
170 sensitive to systemic RAS blockers, and the DOCA-salt rat, a salt- and volume-dependent but
171 renin-independent (low plasma renin levels) model of hypertension resistant to systemic RAS
172 blockers. Both models exhibited an hyperactivity of the brain RAS.
173 Central treatment with the APA inhibitor EC33 blocked the pressor effect of i.c.v AngII in
174 anaesthetized SHRs, suggesting that the increase in BP requires the conversion of AngII into AngIII
175 [54]. The i.c.v injection of EC33 alone leads to an immediate and total inhibition of brain APA activity,
176 thus blocking, in the brain, the formation of AngIII and inducing an decrease in BP in hypertensive
177 DOCA-salt rats and SHRs [32,54] (Figure 3). In contrast, a high intravenous (i.v) dose of EC33 did not
178 modify BP in hypertensive rats, demonstrating that the i.c.v EC33-induced decrease in BP is a
179 central but not due to a systemic effect [32] (Figure 3).

180 Together these findings indicate that AngIII is the effector peptide of the brain RAS. This
181 conclusion was further supported by the significant increase in BP induced by the APN inhibitor
182 PC18, when injected alone, by the i.c.v route, into SHR rats [54]. This pressor response is blocked by

prior injection of the AT1 antagonist losartan (but not by the AT2 antagonist PD 123319), indicating the specificity of action of APN on AngIII metabolism and the involvement of AngIII in the PC18-induced increase in BP. By blocking the action of APN, PC18 causes an accumulation of endogenous brain AngIII, which in turn results in an increase in BP, through interaction with AT1 receptors. Finally, the complete inhibition, by EC33, of the PC18-induced increase in BP demonstrates the existence of the endogenous enzymatic cascade: AngII generated AngIII, itself metabolized in AngIV, mediated by the activities of APA and APN [54].

Consistent with these data, Wright et al. studied the BP effects of AngII and AngIII analogs, D-Asp^{1}AngII and D-Arg^{1}AngIII, which were slightly less degraded than the endogenous corresponding peptides. They injected these compounds, i.c.v into conscious normotensive rats in the presence and absence of EC33 and PC18 [46] and concluded that AngIII was a centrally active ligand of the brain RAS, important in the control of BP. The interpretation of these results was however challenged by Kokje et al. [55], who injected (i.c.v) AngII analogs that were more resistant to aminopeptidase degradation such as N-methyl-L-Asp^1-AngII into conscious normotensive rats. They found that this compound is very slowly degraded and increases BP through AT1 receptor activation. They concluded that AngII, rather than AngIII, is the main active form controlling BP in the brain. However, the finding that i.c.v injection of this analog, which binds AT1 receptors very efficaintly, was expected to increase BP. In fact, in contrast to AngII, which, even in the presence of an APA inhibitor, may be rapidly degraded by other peptidases, such dipeptidyl aminopeptidases, endopeptidases or ACE2, giving rise to Ang3-8 (AngIV), Ang1-5, Ang4-8, and Ang1-7, all being inactive on AT1 receptors, N-methyl-L-Asp1-AngII is only metabolized in Ang2-5. N-methyl-L-Asp1-AngII then remains in major part under its native form, activating AT1 receptors. This underlines the different metabolism profiles of endogenous AngII and exogenous

N-Methyl-L-Asp1-AngII. This illustrates the difficulty to use exogenous metabolically resistant AngII analogs to understand the mechanisms of action of endogenous AngII and AngIII peptides.

Moreover, Wright et al., showed that, despite the high molecular mass of APA and APN (approximately 120–130 kDa for the monomer), i.c.v infusion of APA produces a significant increase in BP [56], whereas i.c.v infusion of APN in SHR rats decreases BP [57]. The pressor effect probably results from a higher level of production of brain AngIII, whereas the hypotensive effect might be related to an increase in AngIII metabolism. Finally, i.c.v infusion of an antiserum that inhibited APA activity reduced the AngII-induced BP increase by 59 % [58].

Together, these studies suggest that brain APA, the enzyme responsible for generating brain AngIII, might be a promising target for hypertension treatment, justifying the development of potent and selective APA inhibitors as centrally-acting antihypertensive agents.

New targets to treat hypertension - Brain Penetrating APA Inhibitor Prodrugs as Centrally Acting Antihypertensive Drugs

While EC33 is an effective centrally acting APA inhibitor, it does not cross the blood brain barrier and hence has limitations for clinical use. However RB150, a prodrug of EC33, was developed and found to be an effective orally active agent [32,33,59]. When given by oral route, RB150 crosses the intestinal, hepatic, and blood-brain barriers and enters the brain. Once in the brain, the disulfide bridge of RB150 is immediately cleaved by brain reductases generating two active molecules of EC33, which inhibit brain APA activity, block formation of brain AngIII and decrease BP and AVP release in conscious hypertensive rats. In alert DOCA-salt rats, the RB150-induced BP decrease is evident 2 h after administration, maximal between 5 and 9 h, and persists after 15 h but without significance; after 24 hours, the drug effect is no longer evident (Figure 4) [60].

The impressive antihypertensive effect of RB150 is attributed to three different mechanisms: (i) decrease in vasopressin release from the posterior pituitary into the blood circulation increased

diuresis and reduced extracellular volume, (ii) decrease in sympathetic tone, decreased vascular resistance and (iii) improved baroreflex function (Figure 5) [33,59,61]. RB150 has no effect on BP in normotensive rats that display hyperactivation of brain aminopeptidase A and brain RAS. Thus, RB150 acts as an antihypertensive agent and not as a hypotensive agent [33,59]. In addition, we showed in conscious SHRs that concomitant oral administration of RB150 with enalapril, an ACE inhibitor, potentiated the RB150-mediated decrease in BP compared with the BP decrease induced by RB150 or enalapril alone [59]. This was especially evident in the acute phase with lower doses. One hour after administration, RB150 at at 100 mg/kg or enalapril at 1mg/kg did not induce any significant BP change, whereas in combination there was a significant BP decrease (-16.4 ± 3.1 mm Hg) [59]. Accordingly we propose that the synergistic effects of combined RB150 with an ACE inhibitor or an AT1 receptor antagonist, may have improved therapeutic effects in the management of patients with hypertension. This may relate to combined inhibition of both the brain and the systemic RAS.

Moreover, oral NI956/QGC006 treatment in hypertensive DOCA-salt rats exhibited a high potency for normalizing brain APA hyperactivity and BP for 10 hours after a single administration, decreasing plasma AVP levels, and increasing diuresis and natriuresis, without affecting plasma sodium and potassium concentrations, at a dose one tenth that required for RB150 [34]. Therefore, NI956/QGC006 is a “best-in-class” central-acting APA inhibitor prodrug, belonging to the same drug class as RB150, supporting the development of antihypertensive therapies targeting brain APA.

These observations not only establish a method for the central delivery of an APA inhibitor but provide insights into the role of brain AngIII in the regulation of vasopressin release and BP in alert DOCA-salt and spontaneously hypertensive rats (SHR). This constituted the first step in the development of a potentially new class of orally active antihypertensive drugs and in 2012, RB150

was selected for clinical development. Toxicology, safety pharmacology and pharmacokinetics studies demonstrated that RB150 is well-tolerated both in rats and dogs up to a dose of 1000 mg/kg during 28 days [59].

Phase I Clinical Trials of Firibastat in Human Volunteers

In a first-in-human study (Phase Ia clinical trial) [62], the safety/tolerability, pharmacokinetics and pharmacodynamic effects of single ascending doses of RB150, renamed firibastat by the World Health Organization, were determined in humans. Healthy male volunteers (n=56) were randomly assigned to receive single oral doses from 10 to 1250 mg of firibastat or placebo. No severe or life-threatening adverse effects were observed. The only treatment-emergent adverse effect considered to be probably related to study treatment was one event of orthostatic hypotension, which occurred in one subject in the 500 mg group. No clinically significant abnormalities were observed in laboratory safety parameters, the haematology panel or clinical chemistry values, vital signs including haemodynamic parameters, or in ECG readings [62]. Pharmacokinetic analysis demonstrated good dose proportional exposure to firibastat and confirmed that once or twice a day oral dosing could be a suitable regimen for further studies. Compared with placebo, firibastat did not significantly change the concentrations of plasma renin, plasma and free urine aldosterone, plasma and urine cortisol, and plasma copeptin, a biomarker of AVP release. No significant change was observed for supine HR, systolic BP and diastolic BP in any of the treatment groups [62]. In a second clinical study (Phase Ib), we confirmed the safety and the tolerability of a single dose up to 2000 mg and multiple oral doses of firibastat up to 750 mg b.i.d. during 7 days in healthy adult subjects (F. Balavoine, M. Azizi, D. Bergerot, N. De Mota, R. Patouret, B. P. Roques and C. Llorens-Cortes unpublished work) (NCT01900171 & NCT01900184). In conclusion, firibastat was shown to be safe and well tolerated following oral administration of ascending

single oral doses up to 2000 mg and repeated oral doses up to 750 mg twice daily for 7 days in healthy subjects.

Phase IIa Clinical Trial of Firibastat in Hypertensive Patients

Following the successful phase I studies, effects of firibastat on BP were assessed in patients. Studies to assess the safety, tolerability, and BP effects by inhibiting brain APA with firibastat were studied for four weeks, in patients with primary hypertension in a phase IIa multicenter double-blind randomized placebo-controlled crossover study (NCT02322450) [63]. Firibastat, at a dose of 250 mg twice daily for one week, with forced titration to 500mg twice daily for three weeks, decreased in the intention-to-treat population (34 patients), daytime ambulatory systolic BP and office systolic BP decreased by 2.7 and 4.7 mmHg respectively vs. placebo, but the difference between the groups was not statistically significant (Table 1). In the per-protocol population (29 patients), in patients with a basal value of daytime ambulatory systolic BP between 154 and 172 mmHg, firibastat treatment induced a larger decrease in daytime ambulatory systolic BP (median [interquartile range (IQR)]: -9.4 [-12.5 to -3.0] mmHg), whereas placebo treatment did not induce any change (median [IQR]: 0.75 [-5.5 to -1.9] mmHg). In the multiple linear regression analysis for the per-protocol population, only treatment with firibastat (P=0.06) and baseline daytime ambulatory SBP (P=0.01) were associated with changes in daytime ambulatory systolic BP but not with plasma renin activity [63]. This suggests that the more the basal daytime ambulatory systolic BP is elevated, the more the firibastat-induced systolic BP decrease is majored [63]. This is in agreement with the observation that, in experimental models of hypertension, firibastat acted as an antihypertensive agent and not as a hypotensive agent. This study showed that brain APA inhibition with firibastat, in patients with mild hypertension, was safe and tended to decrease daytime ambulatory systolic BP relative to placebo at 4 weeks. However, because this was a pilot study, aiming mostly at the safety and tolerability of firibastat, the number of patients involved

was small, and the duration of the treatment too short and the baseline daytime systolic BP not enough high to definitively conclude on the antihypertensive effect of firibastat in humans.

Phase IIb Clinical Trial of Firibastat in Overweight Hypertensive Patients

The results of the Phase IIa study were used to guide the design of a clinical trial Phase IIb, NEW HOPE (NCT03198793) [64]. NEW-HOPE was a multicenter, open-label phase II study in 40 US centers performed in 256 patients overweight or obese (BMI 25-45kg/m^2) hypertensive (systolic BP 145-170 mmHg) patients, including 54% African and Hispanic individuals. After a two-week wash-out period, patients received firibastat for 8 weeks (250 mg twice daily orally for two weeks, then 500 mg twice daily if automated office BP [AOBP] >140/90 mmHg; hydrochlorothiazide 25 mg q.d was added after 1 month if AOPB ≥160/110 mmHg).

The primary endpoint corresponding to change from baseline in systolic automatic office BP (AOBP, SPRINT method) met a significant decrease of 9.5 mmHg [64] ($p < 0.0001$) (Table 1). Systolic AOBP similarly decreased in African-Americans by 10.5 mmHg ($p < 0.0001$) and in Non-Blacks by 8.9 mmHg ($p < 0.0001$) (Table 1). 85% of the subjects did not receive hydrochlorothiazide and were treated with firibastat alone [64]. Most frequent adverse events were headaches (4%) and skin reactions (3%). No angioedema was reported. No change in potassium, sodium and creatinine blood level were observed.

Firibastat may be an attractive potential alternative therapy for African-Americans. In African-Americans, hypertension occurs earlier, is more severe, controlled less often and has a higher morbidity and mortality than in Whites. African-Americans are also less responsive to monotherapy with ACEIs or ARBs [6]. Obesity, higher salt-sensitivity and low plasma renin activity are possible reasons of this poor BP control [65]. The efficiency of firibastat to decrease systolic BP in African-Americans is in agreement with the preclinical studies showing that RB150/firibastat is highly efficient in an experimental salt-dependent model of hypertension with low plasma renin

levels and high plasma vasopressin levels, resistant to systemic RAS blockers [27]. The clinical data on firibastat were recently reviewed by Azizi *et al.*[66].

Conclusions

Growing evidence confirms involvement of the brain RAS in the development of hypertension. Targeting this system with novel agents, such as the first-in-class APA inhibitor prodrug RB150/firibastat has been shown to be very effective. RB150/firibastat crosses the blood-brain barrier after oral administration and thereby inhibits brain APA activity, blocking formation of brain AngIII, one of the main effector peptides of the brain RAS exerting a tonic stimulatory action on BP control in hypertensive rats. This led in turn to a normalization of BP, especially effective in salt-sensitive hypertension. Clinical trials Phase IIa and IIb provide pharmacological proof-of-principle for the efficacy of brain APA inhibition for decreasing BP in hypertensive patients, especially in African-Americans patients, where monotherapy with ACE inhibitors or AT1 receptor antagonists may be less effective. In an era when there are few innovations in antihypertensive drug development, if the proof of concept of firibastat efficacy is confirmed in pivotal Phase III trials, firibastat treatment could provide new hope for improved and better management of hypertension, and may be especially beneficial in subgroups of hypertensive patients, such as those with low renin hypertension, difficult to treat hypertension or resistant hypertension.

Acknowledgments

We thank all of our collaborators for their contributions to work on the aminopeptidase A inhibitors, as well as the members of C. Llorens-Cortes's laboratory, as the organic chemists, Professors B. Roques, M.-C. Fournie-Zaluski, N. Inguimbert, and Doctor E. Chauvel, who designed and synthesized the specific and selective aminopeptidase A and aminopeptidase N inhibitors,

Professor M. Palkovits for his help in neuroanatomical studies and the Quantum Genomics Company.

Sources of Funding

Our own work described in the present paper was supported from the *Agence Nationale de la Recherche* (Emergence, BIOTECS, RPIB CLINAPAI and LabCom CARDIOBAPAI), the *Fondation pour la Recherche Médicale,* the *Institut National de la Santé et de la Recherche Médicale* (INSERM), the *Collège de France* and the Quantum Genomics Company.

Disclosures

C. Llorens-Cortes is co-inventor of the following patents related to aminopeptidase A inhibitors : WO 99/36066 - WO 2004007441 - WO 2005014535, licensed by the Quantum Genomics Company

References

1 GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; **388**: 1659–1724. doi:10.1016/S0140-6736(16)31679-8

2 Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet* 2005; **365**: 217–223. doi:10.1016/S0140-6736(05)17741-1

3 Chobanian AV. Shattuck Lecture. The hypertension paradox--more uncontrolled disease despite improved therapy. *N Engl J Med* 2009; **361**: 878–887. doi:10.1056/NEJMsa0903829

4 Kelly T, Yang W, Chen C-S, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)* 2008; **32**: 1431–1437. doi:10.1038/ijo.2008.102

5 Fryar CD, Ostchega Y, Hales CM, Zhang G, Kruszon-Moran D. Hypertension Prevalence and Control Among Adults: United States, 2015-2016. *NCHS Data Brief* 2017; : 1–8.

6 Flack JM, Sica DA, Bakris G, Brown AL, Ferdinand KC, Grimm RH, Hall WD, Jones WE, Kountz DS, Lea JP, Nasser S, Nesbitt SD, Saunders E, Scisney-Matlock M, Jamerson KA, International Society on Hypertension in Blacks. Management of high blood pressure in Blacks: an update of the International Society on Hypertension in Blacks consensus statement. *Hypertension* 2010; **56**: 780–800. doi:10.1161/HYPERTENSIONAHA.110.152892

7 Smith DHG. Treatment of hypertension with an angiotensin II-receptor antagonist compared with an angiotensin-converting enzyme inhibitor: a review of clinical studies of telmisartan and enalapril. *Clin Ther* 2002; **24**: 1484–1501.

8 Israili ZH, Hall WD. Cough and angioneurotic edema associated with angiotensin-converting enzyme inhibitor therapy. A review of the literature and pathophysiology. *Ann Intern Med*

1992; **117**: 234–242.

9 Owen HG, Brecher ME. Atypical reactions associated with use of angiotensin-converting enzyme inhibitors and apheresis. *Transfusion* 1994; **34**: 891–894.

10 Wright JT, Dunn JK, Cutler JA, Davis BR, Cushman WC, Ford CE, Haywood LJ, Leenen FHH, Margolis KL, Papademetriou V, Probstfield JL, Whelton PK, Habib GB, ALLHAT Collaborative Research Group. Outcomes in hypertensive black and nonblack patients treated with chlorthalidone, amlodipine, and lisinopril. *JAMA* 2005; **293**: 1595–1608. doi:10.1001/jama.293.13.1595

11 Düsing R. Optimizing blood pressure control through the use of fixed combinations. *Vasc Health Risk Manag* 2010; **6**: 321–325.

12 Gradman AH, Parisé H, Lefebvre P, Falvey H, Lafeuille M-H, Duh MS. Initial combination therapy reduces the risk of cardiovascular events in hypertensive patients: a matched cohort study. *Hypertension* 2013; **61**: 309–318. doi:10.1161/HYPERTENSIONAHA.112.201566

13 Jamerson K, Weber MA, Bakris GL, Dahlöf B, Pitt B, Shi V, Hester A, Gupte J, Gatlin M, Velazquez EJ, ACCOMPLISH Trial Investigators. Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. *N Engl J Med* 2008; **359**: 2417–2428. doi:10.1056/NEJMoa0806182

14 Carey RM, Calhoun DA, Bakris GL, Brook RD, Daugherty SL, Dennison-Himmelfarb CR, Egan BM, Flack JM, Gidding SS, Judd E, Lackland DT, Laffer CL, Newton-Cheh C, Smith SM, Taler SJ, Textor SC, Turan TN, White WB, American Heart Association Professional/Public Education and Publications Committee of the Council on Hypertension; Council on Cardiovascular and Stroke Nursing; Council on Clinical Cardiology; Council on Genomic and Precision Medicine; Council on Peripheral Vascular Disease; Council on Quality of Care and Outcomes Research; and Stroke Council. Resistant Hypertension: Detection, Evaluation, and Management: A Scientific Statement From the American Heart Association. *Hypertension* 2018; **72**: e53–e90. doi:10.1161/HYP.0000000000000084

15 Mann SJ. Drug therapy for resistant hypertension: simplifying the approach. *J Clin Hypertens (Greenwich)* 2011; **13**: 120–130. doi:10.1111/j.1751-7176.2010.00387.x

16 Veerasingham SJ, Raizada MK. Brain renin-angiotensin system dysfunction in hypertension: recent advances and perspectives. *Br J Pharmacol* 2003; **139**: 191–202. doi:10.1038/sj.bjp.0705262

17 Sakai K, Sigmund CD. Molecular evidence of tissue renin-angiotensin systems: a focus on the brain. *Curr Hypertens Rep* 2005; **7**: 135–140.

18 Guyenet PG. The sympathetic control of blood pressure. *Nature Reviews Neuroscience* 2006; **7**: 335–346. doi:10.1038/nrn1902

19 Lenkei Z, Palkovits M, Corvol P, Llorens-Cortès C. Expression of angiotensin type-1 (AT1) and type-2 (AT2) receptor mRNAs in the adult rat brain: a functional neuroanatomical review. *Front Neuroendocrinol* 1997; **18**: 383–439. doi:10.1006/frne.1997.0155

20 Wright JW, Harding JW. Important role for angiotensin III and IV in the brain renin-angiotensin system. *Brain Res Brain Res Rev* 1997; **25**: 96–124.

21 Santos RAS, Sampaio WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M, Campagnole-Santos MJ. The ACE2/Angiotensin-(1-7)/MAS Axis of the Renin-Angiotensin System: Focus on Angiotensin-(1-7). *Physiol Rev* 2018; **98**: 505–553. doi:10.1152/physrev.00023.2016

22 Malfroy B, Kado-Fong H, Gros C, Giros B, Schwartz JC, Hellmiss R. Molecular cloning and amino acid sequence of rat kidney aminopeptidase M: a member of a super family of zinc-metallohydrolases. *Biochem Biophys Res Commun* 1989; **161**: 236–241.

23 Vazeux G, Wang J, Corvol P, Llorens-Cortès C. Identification of glutamate residues essential for catalytic activity and zinc coordination in aminopeptidase A. *J Biol Chem* 1996; **271**: 9069–9074.

24 Wu Q, Lahti JM, Air GM, Burrows PD, Cooper MD. Molecular cloning of the murine BP-1/6C3 antigen: a member of the zinc-dependent metallopeptidase family. *Proc Natl Acad Sci*

436 *USA* 1990; **87**: 993–997. doi:10.1073/pnas.87.3.993
437 25 Wilk S, Healy DP. Glutamyl aminopeptidase (aminopeptidase A), the BP-1/6C3 antigen.
438 *Advances in Neuroimmunology* 1993; **3**: 195–207. doi:10.1016/S0960-5428(05)80021-X
439 26 Palmieri FE, Bausback HH, Ward PE. Metabolism of vasoactive peptides by vascular
440 endothelium and smooth muscle aminopeptidase M. *Biochem Pharmacol* 1989; **38**: 173–180.
441 27 Gao J, Marc Y, Iturrioz X, Leroux V, Balavoine F, Llorens-Cortes C. A new strategy for
442 treating hypertension by blocking the activity of the brain renin-angiotensin system with
443 aminopeptidase A inhibitors. *Clin Sci* 2014; **127**: 135–148. doi:10.1042/CS20130396
444 28 Wilk S, Thurston LS. Inhibition of angiotensin III formation by thiol derivatives of acidic
445 amino acids. *Neuropeptides* 1990; **16**: 163–168.
446 29 Chauvel EN, Llorens-Cortès C, Coric P, Wilk S, Roques BP, Fournié-Zaluski MC.
447 Differential inhibition of aminopeptidase A and aminopeptidase N by new beta-amino thiols.
448 *J Med Chem* 1994; **37**: 2950–2957.
449 30 Fournié-Zaluski MC, Coric P, Turcaud S, Bruetschy L, Lucas E, Noble F, Roques BP. Potent
450 and systemically active aminopeptidase N inhibitors designed from active-site investigation.
451 *J Med Chem* 1992; **35**: 1259–1266.
452 31 Réaux A, de Mota N, Zini S, Cadel S, Fournié-Zaluski MC, Roques BP, Corvol P, Llorens-
453 Cortès C. PC18, a specific aminopeptidase N inhibitor, induces vasopressin release by
454 increasing the half-life of brain angiotensin III. *Neuroendocrinology* 1999; **69**: 370–376.
455 doi:10.1159/000054439
456 32 Fournie-Zaluski M-C, Fassot C, Valentin B, Djordjijevic D, Reaux-Le Goazigo A, Corvol P,
457 Roques BP, Llorens-Cortes C. Brain renin-angiotensin system blockade by systemically
458 active aminopeptidase A inhibitors: a potential treatment of salt-dependent hypertension.
459 *Proc Natl Acad Sci USA* 2004; **101**: 7775–7780. doi:10.1073/pnas.0402312101
460 33 Bodineau L, Frugière A, Marc Y, Inguimbert N, Fassot C, Balavoine F, Roques B, Llorens-
461 Cortes C. Orally active aminopeptidase A inhibitors reduce blood pressure: a new strategy

for treating hypertension. *Hypertension* 2008; **51**: 1318–1325. doi:10.1161/HYPERTENSIONAHA.107.098772

34 Keck M, De Almeida H, Compère D, Inguimbert N, Flahault A, Balavoine F, Roques B, Llorens-Cortes C. NI956/QGC006, a Potent Orally Active, Brain-Penetrating Aminopeptidase A Inhibitor for Treating Hypertension. *Hypertension* 2019; **73**: 1300–1307. doi:10.1161/HYPERTENSIONAHA.118.12499

35 Zini S, Masdehors P, Lenkei Z, Fournie-Zaluski MC, Roques BP, Corvol P, Llorens-Cortes C. Aminopeptidase A: distribution in rat brain nuclei and increased activity in spontaneously hypertensive rats. *Neuroscience* 1997; **78**: 1187–1193.

36 Lenkei Z, Palkovits M, Corvol P, Llorens-Cortes C. Distribution of angiotensin type-1 receptor messenger RNA expression in the adult rat brain. *Neuroscience* 1998; **82**: 827–841.

37 Lind RW, Swanson LW, Ganten D. Organization of angiotensin II immunoreactive cells and fibers in the rat central nervous system. An immunohistochemical study. *Neuroendocrinology* 1985; **40**: 2–24. doi:10.1159/000124046

38 de Mota N, Iturrioz X, Claperon C, Bodineau L, Fassot C, Roques BP, Palkovits M, Llorens-Cortes C. Human brain aminopeptidase A: biochemical properties and distribution in brain nuclei. *J Neurochem* 2008; **106**: 416–428. doi:10.1111/j.1471-4159.2008.05402.x

39 Harding JW, Erickson JB, Camara CC, Abhold RH, Wright JW. High-performance liquid chromatographic analysis of ‘specifically bound’ label after [125I]angiotensin II binding to rat brain membranes. *Neurosci Lett* 1986; **65**: 23–28.

40 Harding JW, Felix D. The effects of the aminopeptidase inhibitors amastatin and bestatin on angiotensin-evoked neuronal activity in rat brain. *Brain Res* 1987; **424**: 299–304.

41 Abhold RH, Sullivan MJ, Wright JW, Harding JW. Binding, degradation and pressor activity of angiotensins II and III after aminopeptidase inhibition with amastatin and bestatin. *J Pharmacol Exp Ther* 1987; **242**: 957–962.

42 Checler F. Neuropeptide-degrading peptidases. In *Methods in Neurotransmitter and*

Neuropeptide Research, 1st ed. S.H. Parvez M. Naoi T. Nagatsu, 1993, 375–418.

43 Tieku S, Hooper NM. Inhibition of aminopeptidases N, A and W. A re-evaluation of the actions of bestatin and inhibitors of angiotensin converting enzyme. *Biochem Pharmacol* 1992; **44**: 1725–1730.

44 Zini S, Fournie-Zaluski MC, Chauvel E, Roques BP, Corvol P, Llorens-Cortes C. Identification of metabolic pathways of brain angiotensin II and III using specific aminopeptidase inhibitors: predominant role of angiotensin III in the control of vasopressin release. *Proc Natl Acad Sci USA* 1996; **93**: 11968–11973.

45 Cesari M, Rossi GP, Pessina AC. Biological properties of the angiotensin peptides other than angiotensin II: implications for hypertension and cardiovascular diseases. *J Hypertens* 2002; **20**: 793–799.

46 Wright JW, Tamura-Myers E, Wilson WL, Roques BP, Llorens-Cortes C, Speth RC, Harding JW. Conversion of brain angiotensin II to angiotensin III is critical for pressor response in rats. *Am J Physiol Regul Integr Comp Physiol* 2003; **284**: R725-733. doi:10.1152/ajpregu.00326.2002

47 Murphy TJ, Alexander RW, Griendling KK, Runge MS, Bernstein KE. Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. *Nature* 1991; **351**: 233–236. doi:10.1038/351233a0

48 Karamyan VT, Speth RC. Identification of a novel non-AT1, non-AT2 angiotensin binding site in the rat brain. *Brain Res* 2007; **1143**: 83–91. doi:10.1016/j.brainres.2007.01.051

49 Harding JW, Jensen LL, Hanesworth JM, Roberts KA, Page TA, Wright JW. Release of angiotensins in paraventricular nucleus of rat in response to physiological and chemical stimuli. *Am J Physiol* 1992; **262**: F17-23. doi:10.1152/ajprenal.1992.262.1.F17

50 Saavedra JM. Brain and pituitary angiotensin. *Endocr Rev* 1992; **13**: 329–380. doi:10.1210/edrv-13-2-329

51 Wright JW, Morseth SL, Abhold RH, Harding JW. Pressor action and dipsogenicity induced

514 by angiotensin II and III in rats. *Am J Physiol* 1985; **249**: R514-521.
515 doi:10.1152/ajpregu.1985.249.5.R514
516 52 Campagnole-Santos MJ, Heringer SB, Batista EN, Khosla MC, Santos RA. Differential
517 baroreceptor reflex modulation by centrally infused angiotensin peptides. *Am J Physiol* 1992;
518 **263**: R89-94. doi:10.1152/ajpregu.1992.263.1.R89
519 53 Phillips MI. Functions of angiotensin in the central nervous system. *Annu Rev Physiol* 1987;
520 **49**: 413–435. doi:10.1146/annurev.ph.49.030187.002213
521 54 Reaux A, Fournie-Zaluski MC, David C, Zini S, Roques BP, Corvol P, Llorens-Cortes C.
522 Aminopeptidase A inhibitors as potential central antihypertensive agents. *Proc Natl Acad Sci*
523 *USA* 1999; **96**: 13415–13420.
524 55 Kokje RJ, Wilson WL, Brown TE, Karamyan VT, Wright JW, Speth RC. Central pressor
525 actions of aminopeptidase-resistant angiotensin II analogs: challenging the angiotensin III
526 hypothesis. *Hypertension* 2007; **49**: 1328–1335.
527 doi:10.1161/HYPERTENSIONAHA.107.087130
528 56 Wright JW, Roberts KA, Cook VI, Murray CE, Sardinia MF, Harding JW.
529 Intracerebroventricularly infused [D-Arg1]angiotensin III, is superior to [D-
530 Asp1]angiotensin II, as a pressor agent in rats. *Brain Res* 1990; **514**: 5–10.
531 57 Wright JW, Jensen LL, Cushing LL, Harding JW. Leucine aminopeptidase M-induced
532 reductions in blood pressure in spontaneously hypertensive rats. *Hypertension* 1989; **13**:
533 910–915.
534 58 Song L, Wilk S, Healy DP. Aminopeptidase A antiserum inhibits intracerebroventricular
535 angiotensin II-induced dipsogenic and pressor responses. *Brain Res* 1997; **744**: 1–6.
536 59 Marc Y, Gao J, Balavoine F, Michaud A, Roques BP, Llorens-Cortes C. Central
537 antihypertensive effects of orally active aminopeptidase A inhibitors in spontaneously
538 hypertensive rats. *Hypertension* 2012; **60**: 411–418.
539 doi:10.1161/HYPERTENSIONAHA.112.190942

60 Marc Y, Hmazzou R, Balavoine F, Flahault A, Llorens-Cortes C. Central antihypertensive effects of chronic treatment with RB150: an orally active aminopeptidase A inhibitor in deoxycorticosterone acetate-salt rats. *J Hypertens* 2018; **36**: 641–650. doi:10.1097/HJH.0000000000001563

61 Huang BS, Ahmad M, White RA, Marc Y, Llorens-Cortes C, Leenen FHH. Inhibition of brain angiotensin III attenuates sympathetic hyperactivity and cardiac dysfunction in rats post-myocardial infarction. *Cardiovasc Res* 2013; **97**: 424–431. doi:10.1093/cvr/cvs420

62 Balavoine F, Azizi M, Bergerot D, De Mota N, Patouret R, Roques BP, Llorens-Cortes C. Randomised, double-blind, placebo-controlled, dose-escalating phase I study of QGC001, a centrally acting aminopeptidase a inhibitor prodrug. *Clin Pharmacokinet* 2014; **53**: 385–395. doi:10.1007/s40262-013-0125-y

63 Azizi M, Courand P-Y, Denolle T, Delsart P, Zhygalina V, Amar L, Lantelme P, Mounier-Vehier C, De Mota N, Balavoine F, Llorens-Cortes C. A pilot double-blind randomized placebo-controlled crossover pharmacodynamic study of the centrally active aminopeptidase A inhibitor, firibastat, in hypertension. *J Hypertens* (e-pub ahead of print 14 March 2019; doi:10.1097/HJH.0000000000002092).

64 Ferdinand KC, Balavoine F, Besse B, Black HR, Desbrandes S, Dittrich HC, Nesbitt SD, NEW HOPE Investigators. Efficacy and Safety of Firibastat, a First-in-Class Brain Aminopeptidase A Inhibitor, in Hypertensive Overweight Patients of Multiple Ethnic Origins A Phase 2, Open-Label, Multicenter, Dose-Titrating Study. *Circulation* (e-pub ahead of print 24 April 2019; doi:10.1161/CIRCULATIONAHA.119.040070).

65 Flack JM, Nasser SA, Levy PD. Therapy of hypertension in African Americans. *Am J Cardiovasc Drugs* 2011; **11**: 83–92. doi:10.2165/11586930-000000000-00000

66 Azizi M, Rossignol P, Hulot J-S. Emerging Drug Classes and Their Potential Use in Hypertension. *Hypertension* 2019; : HYPERTENSIONAHA11912676. doi:10.1161/HYPERTENSIONAHA.119.12676

Figure Legends

Figure 1. Schematic diagram of the brain renin-angiotensin system.

ACE: angiotensin I -converting enzyme; APA: Aminopeptidase A; APN: Aminopeptidase N; ACE2: angiotensin-converting enzyme type 2; AT1: angiotensin type 1 receptor; IRAP: Insulin-Regulated-Aminopeptidase.

Figure 2. Distribution of mRNA for the AT1A, AT1B and AT2 receptors and APA in the brain

(A) Distribution of mRNA for the AT1A, AT1B and AT2 receptors in the adult rat brain, sagittal section. (adapted from [19]) - (B) Dark-field photomicrograph of sections showing the distribution of AT1A receptor mRNA in the adult rat brain. The labelling is very high in the subfornical organ (SFO), the vascular organ of the lamina terminalis (OVLT), the median preoptic nucleus (MnPO), the hypothalamic paraventricular nucleus (parvocellular part), the nucleus of the solitary tract (NTS) and the area postrema (AP). Scale bar=1 mm (adapted from [36]) - (C) Distribution of APA enzymatic activity in the adult rat brain. (adapted from [35]). – (D) APA immunoreactivity in the human intermediate medulla oblongata (adapted from [38]) Photomicrographs of human APA immunoreactive somata or fibers in the motor hypoglossal nucleus (XII), the dorsal motor nucleus of the vagus (X), the nucleus of the solitary tract (Sol) and APA immunoreactive vessels (asterisks) in the dorsal motor nucleus of the vagus. (Abbreviations: APit, anterior pituitary; ARC, arcuate nucleus; AT1, angiotensin receptor type 1; AT2, angiotensin receptor type 2; IO, inferior olive; LS, lateral septum; LC, locus coeruleus; LRN, lateral reticular nucleus; MeA, medial amygdaloid nucleus; ME, median eminence; MED, medial cerebellar nucleus; MG, medial geniculate complex; MPO, medial preoptic nucleus; Pir, piriform cortex; PPit, posterior pituitary; PV, periventricular nucleus; R, red nucleus; RF, Reticular Formation; RVLM, rostral ventrolateral medulla; SON,

supraoptic nucleus; Sth, subthalamic nucleus; Th, thalamus; XII, hypoglossal nucleus, X, dorsal motor nucleus of the vagus.

Figure 3. Effects of the APA inhibitor, EC33 on brain APA activity, hypothalamic [^{3}H]AngIII formation and blood pressure.

(A) Time course of the inhibition of brain APA activity after the i.c.v injection of EC33 (100 µg) in alert mice (adapted from [32]) - (B) Percentage [^{3}H]AngIII formation in the hypothalamus 1.5 min after the i.c.v injection of [^{3}H]AngII in alert mice in the presence or absence of EC33 (30 µg) (adapted from [32]) . Values after APA inhibitor treatment were compared with the control values obtained after saline injection - (C) Central and systemic effects of the APA inhibitor EC33 on BP in conscious DOCA-salt rats and SHR (adapted from [32,54]). Mean ± SEM changes in mean BP (ΔMABP in mmHg) following i.c.v injection of EC33 (10–100µg) into alert DOCA-salt rats and SHR and i.v injection of EC33 (45mg/kg) into alert SHR. Mean BP values obtained after the injection of EC33 were compared with the baseline mean BP obtained after the injection of saline. *P<0.05 and **P<0.01, *** P<0.001 *vs.* control values.

Figure 4. Effects of RB150 given by oral route on brain APA activity and blood pressure in alert DOCA-salt rats.

(A) Dose-response inhibition of brain APA activity 3.5 hours after the oral administration of RB150 (7.5 to 50 mg/kg) in conscious DOCA-salt rats or WKY. Mean ± SEM of 3 to 16 animals for each set of conditions. * P<0.05 vs. control values. # P<0.05 vs. DOCA-salt rats non-treated - (B) Mean arterial BP changes in conscious DOCA-salt rats after oral RB150 administration. Peak changes in arterial BP (ΔMABP in mmHg, mean ± SEM) after oral RB150 administration (0.1 to 30 mg/kg) in conscious DOCA-salt rats. (n= 7 for each dose) (adapted from [33]).

Figure 5. Mode of action of the APA inhibitor prodrug, RB150/firibastat, on the control of BP in hypertensive rats.

The brain RAS controls BP via three different mechanisms according to [53]. 1) an increase in vasopressin release from the posterior pituitary into the bloodstream, 2) an activation of sympathetic premotor neuron activity at the level of the rostral ventrolateral medulla (RVLM), and 3) an inhibition of the baroreflex at the level of the nucleus of the solitary tract (NTS). The conversion of AngII into AngIII in the brain involves APA. RB150/firibastat, the prodrug of the APA inhibitor EC33, is composed of 2 molecules of EC33 linked by a disulfide bridge. After oral administration, the disulfide bridge enables RB150/firibastat to cross the blood-brain barrier and to penetrate the brain. In the brain, the disulfide bridge of RB150/firibastat is cleaved by reductases releasing 2 active molecules of EC33, inhibiting APA activity. Consequently, brain AngII is not cleaved into AngIII, which exerts in brain structures a stimulatory action on the control of BP in hypertensive rats. This results in a BP decrease via a decrease in vasopressin release and sympathetic neuron activity. (Abbreviations: APit, anterior pituitary; NTS, nucleus of the solitary tract; PPit, posterior pituitary; PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla; SFO, subfornical organ; SON, supraoptic nucleus. (adapted from [59]).